SafeBridge
CONSULTANTS, INC.

June 15, 1998

C.W. Jameson, Ph.D.
National Toxicology Program
Report on Carcinogens
79 Alexander Drive
Building 4401
P.O. Box 12233
Research Triangle Park, North Carolina 27709

Dear Dr. Jameson:

On behalf of Syntex Agribusiness, Inc., SafeBridge Consultants, Inc., has prepared the enclosed comments setting forth the position that 2,3,7,8-TCDD should be listed as "reasonably anticipated to be a human carcinogen" and not as a "known human carcinogen" in NTP's next Report on Carcinogens. We believe the human evidence is lacking to establish a causal association between TCDD exposure and cancer and that the mechanistic evidence for cancer causation is virtually unknown.

If you are interested in discussing any of our comments, please feel free to call me at (650) 354-7414. We would like to thank the NTP for offering to the general public the opportunity to submit comments.

Sincerely,

Robert H. Ku

Robert H. Ku, Ph.D., DABT, CIH
Principal Toxicologist

enclosure

**Comments**
**in Support of the Position That**
**the Available Evidence for 2,3,7,8-TCDD**
**Supports a Listing of**
**"Reasonably Anticipated To Be A Known Human Carcinogen"**

**Submitted to:**

**National Toxicology Program**
**Report on Carcinogens**
**Research Triangle Park, North Carolina**

**Prepared for:**

**Syntex Agribusiness, Inc.**
**Springfield, Missouri**

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**June 15, 1998**

# COMMENTS
# IN SUPPORT OF THE POSITION THAT
# THE AVAILABLE EVIDENCE FOR 2,3,7,8-TCDD
# SUPPORTS A LISTING OF
# "REASONABLY ANTICIPATED TO BE A KNOWN HUMAN CARCINOGEN"

On behalf of Syntex Agribusiness, Inc., SafeBridge Consultants, Inc., submits the following comments in support of keeping the National Toxicology Program (NTP) classification for 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as *"reasonably anticipated to be a human carcinogen"* rather than a change to *"known to be a human carcinogen"*. As indicated in NTP's listing criteria, for a chemical to be classified as *"reasonably anticipated to be a human carcinogen"*, there must be: (1) sufficient evidence of carcinogenicity from studies in experimental animals, (2) limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias or confounding factors, could not adequately be excluded, or (3) evidence to indicate that it behaves mechanistically similar to a structurally-related class of compounds which already has been classified by NTP as a carcinogen. For a chemical to be classified as *"known to be a human carcinogen"*, there must be sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship, with consideration given to all relevant information, e.g., dose-response, pharmacokinetics, mechanism of action.

We believe the scientific evidence that is currently available for TCDD best supports a classification of *"reasonably anticipated to be a known human carcinogen"*. Based on our review, we believe that the data for TCDD and cancer in humans are limited and inconsistent and that the mechanistic data are weak and support little other than the possibility that TCDD binds to the Ah receptor; whether this step is important to cancer production in animals or humans remains unclear.

## I. The epidemiology studies are equivocal

**A. Historical perspective**

It may be helpful to start by reviewing briefly the history of cancer epidemiology studies and TCDD exposure. In the mid-1980's, most of the "evidence" for human carcinogenicity was based on the studies of Dr. Lennart Hardell and colleagues of herbicide sprayers potentially exposed to TCDD. The cancers associated with potential TCDD exposure that they reported included soft tissue sarcoma (STS) (Hardell and Sandstrom, 1979; Eriksson et al., 1981), non-Hodgkin's lymphoma (Hardell et al., 1981), Hodgkin's disease (Hardell and Bengtsson, 1983), stomach cancer (Axelson et al., 1980), and nasal cancer (Hardell et al., 1982). The relative risks reported in these studies ranged from 2.1 to 6.8. Today, for whatever reasons, these studies are infrequently cited as support for the human carcinogenicity of TCDD.

In the 1990's, the studies by Hardell and colleagues have been replaced by studies of cohorts exposed to TCDD primarily in the manufacture of materials that contained TCDD as an unwanted by-product (e.g., workers in the U.S., Germany, and Netherlands) and a study of a residential cohort in Seveso, Italy, heavily exposed to an environmental release after an industrial accident. These newer studies generally do not confirm the cancer findings reported in the earlier studies but have reported slight elevations of other types of cancers, such as for all cancers combined or lung cancer, with relative risks generally less than 2. Aside from finding such low relative risks, these studies also suffer from various methodological deficiencies and the results may not stand up over time.

**B. Methodological shortcomings**

**1. Exposures to substances other than TCDD**

It should be noted that TCDD is an unwanted by-product of manufactured chemicals (e.g., 2,4,5-trichlorophenol, 2,4,5-trichlorophenoxy acetic acid) and generally was present in low parts per million levels or lower. One of the major shortcomings of the TCDD cancer studies in humans is a lack of recognition that exposure to these manufactured chemicals was exceedingly high, on the order of thousands or tens of thousands of times higher than exposure to TCDD. How certain are we that these manufactured chemicals or products (e.g., 2,4-D; 2,4,5-T, 2,4,5-trichlorophenol) are themselves not carcinogenic in humans?

In a paper published in the Journal of the American Medical Association of farmers in Kansas who used herbicides, Dr. Sheila Hoar of the National Cancer Institute and co-authors stated in the abstract that, "Frequent users who mixed or applied the herbicides themselves had an OR [odds ratio] of 8.0 (95% CI, 2.3, 27.9) for NHL [non-Hodgkin's lymphoma]. Excesses [of NHL] were associated with use of phenoxyacetic acid herbicides, specifically 2,4-dichlorophenoxyacetic acid" (Hoar et al., 1986). It is generally accepted that 2,4-dichlorophenoxyacetic acid does not contain TCDD. In a review paper by Dr. Eric Johnson of the National Institute of Environmental Health Sciences, he stated in the abstract that, "A critical review of the literature indicates that the evidence does not support a causal role for TCDD in the etiology of ML [multiple myeloma]" (Johnson, 1992). In the latest update of the Dutch cohort (Hooiveld et al., 1998), the authors were careful to describe effects observed as associated with "chlorophenols, phenoxy herbicides, and contaminants", not simply with TCDD.

**2. Exposure to other potential carcinogens**

Another major shortcoming of the TCDD cancer studies in humans is that confounding due to exposure to other carcinogenic chemicals either used or made at those facilities where TCDD exposure also occurred was inadequately controlled for. In the study of U.S. workers (Fingerhut et al., 1991a,b; Steenland et al., in preparation), 4-aminobiphenol, a known human bladder carcinogen, was manufactured at one of the 12 plants (Collins et al., 1993). In addition, at least two cases of mesothelioma was reported, indicative of asbestos exposure. At least one case of mesothelioma also was reported in the German BASF workers (Zober et al., 1990). Studies of workers exposed to asbestos who also smoked have been shown repeatedly to have a synergistic effect on the lung cancer rate. In studies of the German Boehringer workers, the authors acknowledged exposure to other materials manufactured at the plant including hexachlorocyclohexane, lindane, dichlorobromophenol, opioids, and dimethyl sulfate (Manz et al., 1991; Flesch-Janys et al., 1995). For this same cohort, Flesch-Janys et al. (1998) reported that exposure to non-TCDD congeners of dioxins and furans represented a larger percentage of workers' exposure than TCDD (TEQ of 130 ng/kg lipid weight basis for non-TCDD congeners vs. 108 ng/kg lipid weight basis for TCDD). IARC (1997) concluded that, "Other polychlorinated dibenzo-para-dioxins are not classifiable as to their carcinogenicity in humans (Group 3)". This conclusion has been reproduced on page 343 of the RC Document.

**3. Confounding due to smoking**

In some studies, smoking was not adequately controlled for (e.g., Fingerhut et al., 1991a,b; Hooiveld et al., 1998). This is a serious flaw because one of the cancer types reported in these studies was a slight elevation in lung cancer. As pointed out above, exposure to both asbestos and smoking greatly potentiates the incidence of lung cancer.

**4. Summary**

In the study of the U.S. cohort involving workers at 12 plants, when plant-by-plant analysis was done, Fingerhut et al. (1991b) reported a significant elevation in lung cancer and all cancers combined at only one plant. Interestingly, in an unpublished study of that plant by Rockette and Arena (1983), workers classified as <u>not</u> exposed to TCDD also exhibited an increase in lung cancer and all cancers combined, suggesting that TCDD may not be the causative agent.

Given the relatively few cancer cases reported in TCDD cancer studies in humans, the attribution of some cases to carcinogenic substances other than TCDD may very well diminish any elevations in lung cancer or all cancers combined reported in these studies. It clearly does not make any sense to attribute all cancer cases to TCDD.

**C. Limitations of environmental epidemiology studies – general considerations**

The perils of interpreting environmental epidemiological studies involving non-infectious agents were described in a Special New Report article in Science (Taubes, 1995). A number of prominent epidemiologists were interviewed, including Dr. Michael Thun of the American Chemical Society, Dr. Ken Rothman, editor of the journal, Epidemiology, Drs. Marcia Angell and Jerome Kassirer, editors of the New England Journal of Medicine, Dr. Dimitrios Trichopoulos, head of the epidemiology department at the Harvard Department of Public Health, and Dr. Robert Temple, director of drug evaluation at the FDA. What do these epidemiologists say about their work?

According to Michael Thun, "With epidemiology you can tell a little thing from a big thing. What's very hard is to tell a little thing from nothing at all."

Dimitrios Trichopoulos asserted that, "We're fast becoming a nuisance to society. People don't take us seriously anymore, and when they do take us seriously, we may unintentionally do more harm than good."

Sir Richard Doll of Oxford University suggested that, "no single epidemiologic study is persuasive by itself unless the lower limit of its 95% confidence level falls above a threefold increase in risk." Harvard's Trichopoulos "opts for a four-fold risk increase as the lower limit." And finally, Robert Temple bluntly stated that, "My basic rule is if the relative risk isn't at least three or four, forget it."

Another problem with "risk-factor epidemiology" is that it is still not standard practice to make available for public review the study protocol before the data are collected. According to Temple, "You always wonder how many ways they cut the data. It's very hard to be reassured, because there are no rules for doing it [that is, making the study protocol available for review prior to data collection]."

As an example, Fingerhut et al. (1991a,b) found a slight increase in risk for all cancers combined in the subcohort with at least one year of exposure to TCDD and at least 20 years of latency.

Was this criterion decided prospectively or "after the data had been cut many ways"? Also, in Flesch-Janys et al. (1995), what was the motivation for the authors to subdivide the TCDD-exposed cohort into quintiles and to further subdivide the highest exposed quintile into two deciles? The relative risks for all cancers combined for the first four quintiles were 1.59, 1.29, 1.66, and 1.60. The relative risks for the two deciles corresponding to the highest exposed quintile were 1.70 and 3.30. Based on this "cut" of the data, a dose-dependent trend for all cancers combined was reported. What is not clear is whether the decision to subdivide the highest exposure quintile into deciles was preconceived, and if not, whether by not subdividng the highest exposed quintile into two deciles a significant trend for all cancers combined would have been found.

**D. Limitations of "best" study associating TCDD exposure and cancer in humans**

The studies frequently cited to support an association between TCDD exposure and increased cancer risk include: (1) a cohort of U.S. workers evaluated by NIOSH (Fingerhut et al., 1991a,b), (2) a Dutch cohort (Buena de Mesquita et al, 1993; Hooiveld et al., 1998), (3) a German cohort of BASF workers in Ludwigshafen (Zober et al., 1990; Ott and Zober, 1996), and (4) a German cohort of Ingelheim workers in Hamburg (Manz et al., 1991; Flesch-Janys et al., 1995, 1998; Becher et al., 1996, 1998). Of these studies, the NIOSH study of workers in 12 U.S. manufacturing plants published in the New England Journal of Medicine (Fingerhut et al., 1991a) is often cited as offering the greatest support for an association between TCDD exposure and cancer.

In addition to Fingerhut et al. (1991a), a companion publication, (Fingerhut et al., 1991b), is available through NTIS. This publication provides additional information on each of the 12 plants not included in Fingerhut et al. (1991a). The NIOSH study is being updated and the manuscript in preparation may have already been submitted for publication (Steenland et al., in preparation). NTP should make sure that this manuscript, once it has been accepted, is included in its review panels' information package, and to make sure that all interested parties are aware of this accepted manuscript. It is our understanding that in last year's review, two accepted, but not yet published, articles (Bertazzi et al., 1997; Hooiveld et al., 1998) were considered by the NTP review panels even though they were not available to the general public.

Below, we point out several limitations in the studies of the NIOSH cohort.

**1. Fingerhut et al. (1991a)**

It may be helpful simply to remind the reviewers what the authors of Fingerhut et al. (1991a) said about their own study in the Abstract:

- Mortality from several cancers previously associated with TCDD (stomach, liver, and nasal cancers, Hodgkin's disease, non-Hodgkin's lymphoma, and soft tissue sarcoma (STS)) was not significantly elevated in this cohort;

- Mortality from "all cancers combined" was significantly elevated (SMR = 1.15);

- In a subcohort with at least one year of exposure and at least 20 years of latency, mortality from STS, respiratory system cancer (SMR = 1.42), and all cancers combined (SMR = 1.46) were significantly increased;

- The study of mortality among workers with occupational exposure to TCDD does not confirm the high relative risks reported for many cancers in previous studies;

- Conclusions about an increase in the risk of STS are limited by small numbers and misclassification on death certificates; and

- Excess mortality from all cancers combined, cancer of the respiratory tract, and STS may result from exposure to TCDD, although we [Fingerhut et al.] cannot exclude the possible contribution of factors such as smoking and occupational exposure to other chemicals.

**2. Fingerhut et al. (1991b)**

Fingerhut et al. (1991b) is essentially a longer version of Fingerhut et al. (1991a) and was published by NTIS. In particular, Fingerhut et al. (1991b) provided more information on body burden levels and cancer cases at each of the individual facilities. The information from Fingerhut et al. (1991b) below obviously also applies to Fingerhut et al. (1991a).

- Fingerhut et al. (1991a,b) included 5,172 workers from 12 plants in the U.S.; 253 workers from 2 of the 12 plants were included in the body burden study; (the next two points below apply only to these 253 workers (less than 5% of the entire cohort));

- At the time of the study, on a lipid basis, the mean TCDD body burden was 233 ppt and the maximum was 3,400 ppt; the group with less than 1 year of exposure and greater than 20 years of latency was 78 ppt, and the group with greater than 1 year of exposure and greater than 20 years of latency was 462 ppt;

- For half-life extrapolated TCDD body burdens to the year of last occupational exposure, on a lipid basis, the mean was 2,000 ppt and the maximum was 32,000 ppt; the group with less than 1 year of exposure and greater than 20 years of latency the TCDD body burden was 640 ppt, the group with greater than 1 year of exposure and greater than 20 years of latency the TCDD body burden was 3,600 ppt;

- The subcohort (taken from the entire cohort of 5,172 workers) with over 1 year of exposure and over 20 years of latency was exposed to TCDD-contaminated processes for 6.8 years on average and to non-TCDD-contaminated processes for 12.4 years on average (Table 5);

- The four STS cases occurred at 2 plants (plants 8 and 9), two at each plant, and the rate was considered significant at one of the plants (Table 6);

- In only one plant (plant 10) were the SMR for lung cancer and all cancers combined significantly elevated (Table 6);

- No other significant elevation was evident, by plant, for cancers of a priori interest (STS, non-Hodgkin's lymphoma, Hodgkin's disease, stomach, and liver), lung cancer, and all cancers combined (Table 6).

**3. Steenland et al. (in preparation)**

Steenland et al. (in preparation) updates Fingerhut et al. (1991a,b) by adding 6 more years of follow-up. In short, the relative risks reported for the cancer categories of concern appeared to have either remained the same or decreased. One novel aspect of Steenland et al. (in preparation) was the use of a cumulative exposure matrix scoring system to estimate worker exposure to TCDD. This exposure matrix was described in a poster at the Dioxin '97 meeting in Indianapolis and also at the 1998 American Industrial Hygiene Conference and Exposition in Atlanta. Interestingly, the mean exposure matrix score varied by over 4 orders of magnitude from plant to plant. The differences in estimated TCDD exposures for workers from plant to plant suggests that exposures to other chemicals (during the same time frame of interest) may have varied by just as much. Also, as pointed out in the fourth bullet in the preceding section, workers in the U.S. cohort worked an average of 12.4 years with non-TCDD-contaminated processes as compared to an average of 6.8 years with TCDD-contaminated processes. Further, the employment histories of these workers before they started working at these 12 plants are unknown. Thus, these limitations point to the difficulties in trying to interpret results from studies of this cohort.

**4. Limitations With NIOSH Study**

The limitations of the NIOSH study have been well articulated by the authors themselves and shown above. Those limitations are expanded on below:

- The SMRs for all cancers combined (SMR=1.46) and respiratory system cancers (SMR=1.42) found were modest. Epidemiologists, Drs. Richard Doll, Dimitrios Trichopoulos, and Robert Temple, voiced their opinion in a recent article in Science that for environmental epidemiology studies such as Fingerhut et al. (1991a,b), the relative risk should be 3 or 4 before one should take note.

- For the category, all cancers combined, all of the cancer cases should not be attributed to TCDD. Collins et al. (1993) pointed out that 4-aminobiphenyl, a known human bladder carcinogen, was made at one of the 12 plants; mesothelioma cases should have been more properly attributed to asbestos exposure; the effect of asbestos exposure on lung cancer rates in smokers need to be considered; some lung cancer cases should have been attributed to smoking, etc.

- Substantial differences existed from plant to plant in the: (1) duration of exposure to TCDD-contaminated processes, (2) magnitude of exposure to TCDD, (3) opportunity for exposure to chemicals other than TCDD, etc. (It appears that nothing is known about these individuals' work history before they started working at the 12 plants.)

- The category, all cancers combined, is fairly unique. The simplest and more likely explanation for a finding of all cancers combined would be that these cancers were produced by different chemicals or factors. When one compares Fingerhut et al. (1991a,b) with other recent studies, one discovers that not all studies reported an increase in all cancers combined or in lung cancer. If these reported elevations are indeed real, the spectrum of cancers reported appears to be vary from study to study, once again suggestive of multiple causative agents. Finally, the spectrum of cancers reported in humans differs from the spectrum of cancers produced in laboratory animals. This observation should raise one's concern over the reliability of using animal data to predict human cancer both qualitatively and quantitatively and this point is described in more detail later in these comments.

**E. Studies of Seveso Cohort**

The cancer mortality studies of the Seveso, Italy, cohort have been reported in Bertazzi et al. (1993, 1997) and appear to show no discernable pattern comparable to the occupational cohort studies. In particular, as stated in the abstract of Bertazzi et al. (1997), "We [authors] found no increase in all-cancer mortality or major specific sites (for example, respiratory among males, breast among females)."

Recently, Needham et al. (1998) presented body burden data of cancer cases in Zone A (the most highly contaminated zone in Seveso). The mean body burden of these 9 cases was 303 ng/kg (lipid weight basis). This mean body burden level is lower than the mean body burden level of 443 ng/kg (lipid weight basis) for Zone A residents reported in Bertazzi et al. (1997), suggesting that either TCDD body burden is not a good predictor of cancer in humans or that TCDD is unlikely to be the causative agent for these cancers. [Interestingly, in a study by Hardell and colleagues in the 1980's of herbicide sprayers (as cited by EPA, 1988), the TCDD body burdens of individuals with STS was found to be the same as for controls, again suggesting that either TCDD body body burden is not a good predictor of cancer in humans or that some other etiological agent was responsible for the STS.]

One of the limitations frequently cited of Bertazzi et al. (1993, 1997) has been that the latency period has not been long enough. These studies reported findings for a time period of 10 and 15 years after the accident, respectively. Mechanistically, there is ongoing debate as to whether TCDD acts as a tumor promoter or initiator. Tumor promoters are defined as agents that do not cause genetic damage but can promote the genetic damage to subsequent tumor formation after some genetic damaging event has already occurred; a very short latency period is necessary for tumor formation. Tumor initiators are defined as agents that cause genetic damage; a long latency period is necessary for tumor formation. Most of the evidence suggest that TCDD act as a tumor promoter (Lucier et al., 1993; Whysner and Williams, 1996). If TCDD acts as a tumor promoter in humans, then the latency period in studies of the Seveso cohort or in any of the occupational cohort studies would not be such a big factor in the design of the study or in the interpretation of the data. The recent occupational cohort studies emphasize substantially the need for a long latency period, clearly at odds with our current mechanistic understanding of TCDD-induced carcinogenicity in animals.

Thus, based on the reported body burden data for cancer cases, the types of cancers reported, and mechanistic considerations, the studies of the Seveso cohort are inconsistent with TCDD as the cancer causing agent.

**II. The carcinogenic mechanism of action of TCDD in animals is unknown**

There appears to be consensus that most of the adverse effects of TCDD are associated with TCDD binding to the Ah receptor. It also is recognized that some of the adverse effects of TCDD are not related to TCDD binding to the Ah receptor. And the jury is still out as to whether cancer in animals is mediated via TCDD binding to the Ah receptor. In the Lucier et al. (1993) review, the authors stated that, "[m]ost, if not all, of TCDD's toxic and biochemical effects, including tumor promotion, are Ah receptor dependent". However, the authors also stated that, "there are likely multiple mechanisms of tumor promotion" and that "each of these mechanisms may be fundamentally different from the others". The authors proceeded to describe several Ah receptor-dependent tumor promotional mechanisms. But the fact remains that we do not know whether any of these mechanisms are related to the cancer that is subsequently produced in animals.

Some insight may be obtained from the carcinogenicity of chemicals related to TCDD. A mixture of two congeners of hexachlorodibenzo-p-dioxin (HCDD) was tested in two-year cancer bioassays in the mouse and rat. Based on the dose administered and the cancer rate observed, it was determined that HCDD was 1/20 as potent a liver carcinogen as TCDD (Lucier et al., 1993). When Poland et al. (1976) and Safe (1990) evaluated Ah receptor binding potency using hepatic cytosol obtained from the mouse and rat, they found the binding affinity of HCDD to be approximately one-fifth and one-fourth as potent as TCDD, respectively. These data would suggest that either other steps are involved in the carcinogenicity of these compounds in animals in addition to binding to the Ah receptor or that binding to the Ah receptor is not related to carcinogenicity.

Recently, Pollenz et al. (1998) reported that female rats exposed to a single oral dose of TCDD exhibited a sustained depletion of the Ah receptor in liver, spleen, thymus, and lung. It is not known whether this effect also occurs in humans. Any toxicities mediated by the Ah receptor in animals or humans could be profoundly influenced by this effect.

**III. The carcinogenic mechanism of action of TCDD in humans is unknown**

There is little or no information available on the carcinogenic mechanism of action of TCDD in humans. All that is known is that humans, similar to animals, also possess the Ah receptor. However, the steps subsequent to the binding of TCDD to the Ah receptor in animals and the ultimate manifestation of cancer is unknown. Responses in different tissues in animals and humans are believed to be dependent on the myriad of gene transcription products that are responsive to TCDD (Whysner and Williams, 1996).

One piece of evidence arguing against a role for the Ah receptor in human cancer comes from a subgroup of the U.S. cancer mortality cohort of Fingerhut et al. (1991a,b). This subgroup consisted of a subgroup of workers from 2 of the 12 plants evaluated in Fingerhut et al. (1991a,b) which was evaluated for CYP1A2 enzyme induction (Halperin et al., 1995). CYP1A2 induction was not found in these workers even though enzyme induction is considered to be one of the most sensitive Ah receptor-dependent biological effects of TCDD. The mean TCDD body burden level in this subgroup of workers was reported to be 157 ng/kg (lipid weight basis) with a maximum individual of 1,742 ng/kg (lipid weight basis).

While there is little or no evidence to suggest that humans would respond differently to animals to the carcinogenic effects of TCDD, it would be a stretch to invoke this line of scientific reasoning to support a common mechanism across species.

## IV. Human exposures in epidemiological studies are not comparable to animal exposures in carcinogenicity studies

Presumably, in support of reclassifying 2,3,7,8-TCDD as a known human carcinogen, the RC Document states on page 3-1 that, "the tissue concentrations of TCDD were similar in heavily exposed human populations (where an increased cancer risk was observed) and in rats exposed to carcinogenic dosages (DeVito et al., 1995)." First of all, it is not clear how anyone would conclude that a body burden range of 109 to 7,000 ng/kg body weight [in humans] can even be considered comparable to a body burden range of 944 to 137,000 ng/kg body weight [in animals]. Several issues need to be considered before one can determine if the animal and human data for cancer are mutually supportive of each other.

### A. Is body burden (in units of ng/kg (body weight basis), as cited in DeVito et al. (1995)) a good dosimetric for predicting cancer across species?

There is no question that TCDD produces tumors in animals. It is evident that the sites where cancer has been reported in humans are different from the cancer sites of TCDD-treated animals. Further, it is unclear how TCDD causes cancer. One plausible scenario is that TCDD levels at some target tissue(s) (which may or may not be the same tissue(s) as where the tumors manifest) may be important. There is no way of telling whether the TCDD levels at critical target tissues are related to the overall body burden of TCDD (in units of ng/kg body weight). Thus, even if the overall body burden is comparable across species, there is no evidence to indicate that the level in the liver, or in any other tissue, is comparable across species. Furthermore, it should be noted that the cancer incidence rates in animals and humans of the studies cited in DeVito et al. (1995) were substantially different. For example, in the two-year cancer bioassay study in the rat (Kociba et al., 1978), most of the female rats developed liver tumors at the highest dose administered of 100 ng/kg/day. In contrast, in the Fingerhut et al. (1991a) study of U.S. workers, the SMR for all cancers combined was only 1.15.

To look at this issue in another way, it is clear that in Kociba et al. (1978), the high dose administered to the rat was 100 ng/kg/day. If this dose were administered to humans, it would result in a body burden of about 400,000 ng/kg body weight (assuming first order kinetics and a 7 year elimination half life). No human has ever experienced a body burden of this magnitude. In contrast, DeVito et al. (1995) presented a body burden in the rat dosed at 100 ng/kg/day of about 3,000 ng/kg body weight.

Thus, from this analysis, one cannot conclude that the body burden for producing cancer in humans and animals are comparable. The animal and human evidence are consistent if one concludes that the reason why TCDD has not been definitively linked to cancer in humans is because human exposures have not been high enough.

**B. The body burden levels cited in DeVito et al. (1995) are misleading and erroneous**

For the range of body burdens from 109 to 7,000 ng/kg body weight suggested to be associated with cancer in humans, DeVito et al. (1995) cited an occupational cohort (Fingerhut et al., 1991a) and the Seveso cohort (Bertazzi et al., 1993). The Fingerhut et al. (1991a) study is a study of workers at 12 different facilities across the U.S. Actual body burden measurements (lipid weight basis) were obtained only from workers in 2 of the 12 facilities and the body burdens of workers in the other 10 plants were inferred. Also, because body burden measurements were taken years after occupational exposure had ceased, an extrapolation was done to estimate the body burdens of each individual (in the 2 facilities) at the time of last occupational exposure and inferred for workers at the other 10 plants.

There is insufficient information presented in Fingerhut et al. (1991a) to allow a calculation of a body burden associated with the subcohort exhibiting a slight elevation in cancer risk. Presumably based on additional information, EPA (1996) performed this calculation and came up with 354 ng/kg body weight for this subcohort. According to EPA (1996), the half-life extrapolated body burden range (to the year of last occupational exposure) (again only for workers in 2 of the 12 facilities) was from 2,000 to 32,000 ng/kg (lipid weight basis). The subcohort who was exposed greater than one year and with greater than 20 years latency reported by Fingerhut et al. (1991a) to exhibit a slight increase in cancer had a median body burden of 1,770 ng/kg (lipid weight basis). [Fingerhut et al. (1991b) reported a mean body burden of 3,600 ng/kg (lipid weight basis) for this subcohort]. Assuming a body weight of 70 kg and 20% adipose tissue, this body burden is equivalent to 354 ng/kg (body weight basis). EPA (1996) estimated that this body burden level is achieved by an average oral daily dose of 63 pg/kg/day.

The high end of the range presented in DeVito et al. (1995) of 7,000 ng/kg body weight was based on Bertazzi et al. (1993). However, Bertazzi et al. (1993) does not provide body burden levels for the Seveso cohort. In Bertazzi et al. (1997), body burden levels in Zones A, B, and R (corresponding to the high, medium, and low contamination areas in Seveso) of 443, 87, and 15 ng/kg (lipid weight basis) were given for individuals older than 13 years at the time of the accident. In light of these new data, the 7,000 ng/kg (body weight basis) (or 35,000 ng/kg (lipid weight basis, assuming 70 kg body weight and 20% lipid)), should be replaced by 89 ng/kg (body weight basis) or 443 ng/kg (lipid weight basis). Our analysis would indicate that the body burden range of humans reported to have a slight elevation of cancer should be approximately 89 to 354 ng/kg body weight, rather than the 109 to 7,000 ng/kg (body weight basis) range reported in DeVito et al. (1995).

For animals, DeVito et al. (1995) cited a body burden value in the mouse, rat, and hamster of 944, 2,976, and 137,000 ng/kg body weight. These values were not measured but calculated after making several pharmacokinetic assumptions. The validity of any of these assumptions, particularly after long-term exposure, is not known. For the study by Kociba et al. (1978) in the rat, body burden measurements were actually taken but apparently not used in DeVito et al. (1995). At the 100 ng/kg/day administered dose, the body burden was 8,100 ng/kg (lipid weight basis). Assuming that at the end of the two-year cancer bioassay the rats weighed 0.5 kg and 20% was lipid weight, then the body burden would have been 1,620 ng/kg (body weight basis) (as compared to the 2,976 ng/kg (body weight basis) value reported in DeVito et al. (1995)).

Based on these calculations, the body burden levels needed to produce cancer in animals appear to be higher than in humans. If the use of body burdens is predictive of cancer, this conclusion is consistent with the cancer data in animals and humans. Whereas increased cancer rates in animal studies were obvious, they were clearly the result of much higher body burden levels. The cancer studies in humans are equivocal because exposures (and resultant body burden levels) have not been high enough to demonstrate this effect definitively. Thus, the question of whether TCDD can cause cancer in humans remains unproven.

**C. Is the TCDD body burden level in the liver a better predictor of cancer in animals and humans than overall TCDD body burden?**

It is recognized that TCDD is a tumor promoter (Lucier et al., 1993; Whysner and Williams., 1996). In the two-year cancer bioassay in the rat, Kociba et al. (1978) suggested that a plausible cause for the observed elevated rate of liver cancer was because of persistent liver toxicity and increased liver cell turnover caused by TCDD. Curiously, at the mid- and high-dose of 10 and 100 ng/kg/day, the amount of TCDD in the liver was 3 times higher than the level of TCDD in the fat (5,100 ng/kg in the liver vs. 1,700 ng/kg in the fat at the 10 ng/kg/day dose and 24,000 ng/kg in the liver vs. 8,100 ng/kg in the fat at 100 ng/kg/day dose). At the low dose of 1 ng/kg/day where cancer rate was not elevated, the level of TCDD was 540 ng/kg in both the liver and fat. In humans, there does not appear to any published data on TCDD levels in the liver in highly exposed groups. As mentioned earlier, enzyme induction was not found in a subgroup of workers from the Fingerhut et al. (1991a,b) cohort (Halperin et al., 1995). In a study of liver toxicity which included this subgroup and other workers from the Fingerhut et al. (1991a,b) cohort, little evidence of TCDD-induced liver toxicity was found (Calvert et al., 1992). In humans exposed to background levels of TCDD, the level of TCDD in the fat and liver is comparable (lipid weight basis). It has been hypothesized that when exposure is high enough to change the ratio, i.e., to high in liver relative to fat, then cancer could be produced (Ku et al., 1991). This level of exposure occurred in the rat, resulting in the excess of liver cancer reported in Kociba et al. (1978). This level of exposure apparently has not occurred in humans since an excess in liver cancer has not been reported.

**D. Is TCDD administered dose a better dosimetric than TCDD body burden for predicting cancer in animals and humans?**

The determination of body burden values in animals and humans require making a number of pharmacokinetic assumptions. If one were to use the administered dose, no assumptions are necessary. As presented in DeVito et al. (1995), in the mouse, rat, and hamster, the administered dose is 71, 100, and 100,000 ng/kg/day. As calculated in EPA (1996), the median administered dose in the Fingerhut et al. (1991a) subcohort reported to have an increase in cancer was 0.063 ng/kg/day. If this is the appropriate dosimetric to use for cross-species comparison, then it is clear why TCDD is the unlikely cause of cancer in this subcohort since the TCDD dose in this subcohort is more than 1000 times lower than the TCDD dose which caused cancer in animals. Similar to the use of body burdens, however, there is no evidence to suggest that administered dose is the appropriate dosimetric to make cross-species comparisons.

**E. Other dosimetrics have been proposed for cross-species comparison**

Aylward et al. (1996), Flesch-Janys et al. (1998), and Becher et al. (1998) proposed the use of area-under-the-curve (AUC) as another alternative for cross-species comparison. At this point, whether this dosimetric is any better than any of the other ones described above remains unsubstantiated.

**F. Summary**

Presumably, the reason the RC Document stated that the TCDD tissue concentration in heavily exposed humans (where an increase in cancer was reported) and in rats exposed to carcinogenic doses of TCDD was to demonstrate that humans and animals behave similarly at similar TCDD tissue concentrations, and thus to lend support to classifying TCDD as a known human carcinogen. However, when one carefully looks at how those tissue concentrations were calculated, one finds that either there was insufficient information in the original articles to make those calculations or that the calculations were done incorrectly. More importantly, the RC Document does not justify why the use of body burdens (as opposed to some other dosimetric) would be a good predictor of cancer in animals and humans. TCDD carcinogencity is believed to be multifactorial and overall body burden levels may, in fact, poorly predict cancer in animals and humans. Other dosimetrics may be more plausible, e.g., liver body burdens, administered dose, AUC. If animals and humans are equally sensitive, one plausible conclusion could be that human exposures have been insufficient to produce cancer and that the slight cancer excesses reported in humans, if real, are not attributable to TCDD.

**V. Based on animal carcinogenicity studies, it is scientifically justified to support the position that, if comparably exposed, humans will develop cancer**

There is little doubt that TCDD causes cancer in animals whereas the data in humans are still questionable. There is no evidence to suggest that humans would be resistant to TCDD-induced cancers. The most likely reason why the evidence in humans is uncertain is because exposures to humans have not been high enough. This can be seen in our calculations above, i.e., that when either the administered dose or body burden is used in comparing TCDD-induced cancer in animals and humans. It is likely that if exposures to humans were higher, then humans will most likely develop cancer. Thus, based on what we know today, the most appropriate classification for TCDD is as an anticipated human carcinogen.

If data do become available to substantiate a causal association between TCDD and cancer in humans, it is likely, based on what we know today, that this causal relationship would apply only to the most highly exposed group of individuals. Consideration should be given to the fact that anyone today experiencing exposures as high as in these most highly exposed group of individuals is improbable. Current concerns are with exposures to environmental levels of TCDD. These exposure levels are several orders of magnitude lower than that experienced by the most highly exposed group of individuals. Furthermore, environmental concentrations have been decreasing due to discontinuation and/or reduced production of certain chemicals, process changes in the pulp and paper industry, and more stringent emissions requirements for incinerators. It is important to consider these issues in the event that TCDD is classified as a known human carcinogen. Not only do the humans studies to date indicate a "threshold" for cancer (if there effects are indeed real), our current understanding of the mechanism of action of TCDD also supports a threshold mechanism.

## VI. The issue of toxicity equivalence factors (TEFs)

There has been a great deal of discussion and debate as to whether dioxin-like congeners other than TCDD should to be treated like TCDD, using an approach such as the toxicity equivalence factor (TEF) approach (EPA, 1989; Ahlborg et al., 1992, 1994). The NTP is urged to explicitly make a statement that its classification is only for TCDD and that the utilization of this classification for dioxin-like compounds is inappropriate. This was the position appropriately taken by IARC (1997).

## VII. The classification of 2,3,7,8-TCDD should remain "anticipated"

Based on a review of the human TCDD literature, we conclude that there is inadequate evidence to support the position that TCDD is causally associated with cancer in humans. The studies cited in support of an association are limited by study design flaws and the inability to dismiss the contribution of confounding exposures to other carcinogenic agents and for smoking. The relative risks reported are essentially all less than 2. Epidemiologists such as Drs. Richard Doll, Dimitrios Trichopoulos, and Robert Temple believe for these types of studies the relative risks had to be at least 3 or 4 before they should be considered seriously. The IARC epidemiology subpanel voted to keep the classification for TCDD the same, which evidently was overruled by the full panel based on mechanistic considerations. However, when one looks at the mechanistic evidence for cancer in animals or humans, one finds that there is very little known other than the fact that TCDD binds to the Ah receptor. It is not even clear if this initial step is critical to the subsequent production of cancer in animals. Even less is known about the mechanism in humans. Because the evidence for cancer in animals is convincing, and because there is no evidence that humans are expected to respond differently to TCDD than animals, the most appropriate classification for TCDD would be keep TCDD as an anticipated human carcinogen. It is plausible that human exposure to TCDD of the same magnitude (if we knew what the most appropriate dosimetric to use for cross-species comparison) as animals would cause cancer. The weight of scientific evidence supports the position that under current exposure scenarios no one will be exposed to TCDD levels (by a margin of 3 or 4 orders of magnitude) high enough to produce cancer.

## VIII. References

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